Cell type specific expression of tumor necrosis factor-a in the CNS and pituitary of transgenic mice

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275

Proinflammatory Cytokines, Neurotrophic Factors, and Reactive Astrogliosis during CNS Trauma

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Following injury to the adult CNS, astrocytes become reactive and produce neurotrophic factors in what appears to be a failed attempt to promote recovery. Identification of the molecular mediators of this process known as astrogliosis is necessary in order to establish suitable conditions for axonal regeneration and remyelination. In a corticectomy model of injury in adult mice, proinflammatory cytokines IL-1 and TNF-α mRNA levels become elevated within 1 hour as measured by RT-PCR. By in situ hybridization, IL-1 is localized in a specific population of cells around the injury site and within the corpus callosum. This elevation of proinflammatory cytokines precedes the rise in GFAP mRNA, the earliest discernible manifestation of astrogliosis, and the upregulation of neurotrophic factor transcripts such as CNTF. Current experiments test the hypothesis that proinflammatory cytokines activate astrocytes to become reactive and influence their production of neurotrophic factors.

276

Expression of the Anaphylatoxin C5a Receptor by Glial Cells and T Lymphocytes in Experimental Allergic Encephalomyelitis

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In this study, we investigated the expression of the C5aR in spinal cords of Lewis rats with experimental allergic encephalomyelitis (EAE). Using in situ hybridization (ISH) we analyzed the kinetics of C5aR expression at different time points of EAE (preclinical stage, clinical peak, remission phase). While C5aR mRNA was constitutively expressed at variable low levels in neurons, some blood vessels and a subpopulation of astrocytes in healthy control rats, it was readily detected in inflammatory cells invading the CNS at all the stages of EAE. Using a combination of ISH and immunohistochemistry we identified multiple cell types expressing the C5aR in the CNS of EAE rats. Based on cell morphology the C5aR mRNA was localized in activated microglial cells and round ED1-positive cells corresponding to monocyte/macrophages. In addition, hypertrophic astrocytes strongly expressed both C5aR mRNA and GFAP particularly during disease remission. Surprisingly, C5aR mRNA was also detected in infiltrating T lymphocytes recognized by an anti-TCR antibody. The potential involvement of C5a and its receptor in cell trafficking and activation of CNS immunocompetent cells is discussed.

277

Inhibition of IFN-gamma Induced Class II Transactivator and Class II MHC Expression in Microglia

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Microglia are the brain's resident macrophage and when activated have functions including cytokine production and antigen presentation. The class II genes in the major histocompatability complex (MHC) locus encode proteins that present antigen to CD4+ T-cells, leading to their activation and the development of an antigen specific immune response. In microglia, class II MHC expression is upregulated by interferon-γ (IFN-γ). Class II MHC gene expression is controlled by the class II transactivator (CIITA) transcription factor. IFN-γ induced expression of the CIITA gene is controlled by one its four promoters (Promoter IV). In this study, we investigated the effects of TGF-β1, IL-4, and IL-10 on IFN-γ induced class II MHC and CIITA expression. By FACS analysis, we show that IFN-γ induced class II MHC protein expression is down-regulated by TGF-β1, IL-4 and IL-10. Using a RNase protection assay, we show that TGF-β1, IL-4 and IL-10 inhibit CIITA mRNA and in turn class II MHC mRNA expression. Studies are ongoing to understand the molecular mechanisms underlying the inhibitory effects of these cytokines.

278

Site Specific Immune Regulation in the CNS Revealed by Low-dose IFN-gamma

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Although neurotransmitters and neuropeptides are known to affect immune function in vitro, little is known about how the local mix of neurochemicals influences immune activity in the CNS. Using a novel model, we studied local modulation of the immune response. Specifically, T cell traffic and MHC expression were examined in two sites with very different neurochemical environments, the brain stem and hippocampus. The cytokine gamma interferon (IFN-g, 0.1 to 10,000 U / site) was injected stereotaxically into the hippocampus and contralateral brain stem of adult CDF rats. Two or four days later, monoclonal antibody staining was used to detect class I and II MHC proteins or T cells on cryostat sections, followed by quantitative computer-assisted image analysis. As compared to the hippocampus, the brain stem showed enhanced MHC expression by microglia at lower IFN-g doses, and reached a higher plateau. Site-specific MHC modulation was also seen within the layers of the hippocampus, and among other brain sites. Moreover, different patterns of T cell migration into the parenchyma were observed in the two CNS regions. Our preliminary data suggests that the local neuroregulatory environment, such as the high SP levels in the brain stem, contributes to this site-specific control. These findings have implications for the therapeutic control of the immune response, and may help explain the localization of pathological neuro-immune conditions such as MS plaques.

279

Differential Effects of Interleukin-4 and Interleukin-10 on IL1.β Induced Mouse Primary Astrocyte Activation Comparison with Dexamethasone

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The inflammatory cytokine IL-1β is able to induce severe cellular brain damage. Different processes regulate IL-1 biological activities, like the production of anti-inflammatory cytokines, e.g. IL-4 and IL-10. We describe, here, the effects of IL-4 and IL-10 on IL-1β-induced IL-6 production in mouse primary astrocytes and compare these effects to those of dexamethasone. IL-6 secretion and mRNA expression were determined by ELISA and RT-PCR respectively. Simultaneous stimulation of cells with IL-1β+IL-10 or IL-1β+dexamethasone for 6 h markedly reduced IL-1β induced IL-6 secretion and IL-6 mRNA expression respectively, whereas simultaneous addition of IL-4 had no effect. In contrast, after 24 h of IL-1β pretreatment, IL-6 production was decreased below constitutive levels, and this change was reversed by addition of IL-4. The delayed effect of IL-4 might be partially explained by the induction of IL-4 receptor α-chain mRNA expression by IL-1β. We conclude that IL-10 and dexamethasone are rapid inhibitors of IL-6 in IL-1β activated astrocytes, whereas IL-4 stimulates IL-6 production. These results suggest that IL-4 and IL-10 play an essential role in the regulation the cerebral inflammatory response.

280

Cell Type Specific Expression of Tumor Necrosis Factor-a in the CNS and Pituitary of Transgenic Mice

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Tumor necrosis factor-α (TNF-α) plays a central role in inflammatory processes including those of autoimmune diseases of the CNS. To investigate cell type specific functions of TNF-α in the CNS and in the pituitary we have cloned and characterized novel neuronal promoters (NMDAR subunits ε2 and ε3) and used these together with the engrailed-1 and ratPOMC promoter to generate transgenic and knock-in mice. Analyses of transgene expression and the resulting phenotypes are currently performed. A transgenic mouse line expressing murine TNF-α under control of the ratPOMC promoter shows restricted transgene expression in the intermediate lobe of the pituitary resulting in CD3-positive cellular infiltration. Expression in the intermediate lobe can be upregulated by application of the dopamine antagonist Haloperidol. Adrenalectomy results in strong transgene expression in the anterior lobe. These data demonstrate the neuronal and hormonal regulation of TNF-α transgene expression in the pituitary and the activation of the hypothalamus-pituitary-adrenal axis. In conclusion, the use of cell type specific promoters for targeting the expression of TNF-α in defined cellular subsets opens an approach to investigate both local cytokine functions and systemic reactions thereof.